

**REPRODUCTIVE DIFFERENCES BETWEEN *RHAGOLETIS*
(DIPTERA: TEPHRITIDAE) FRUIT PARASITES OF
CORNUS AMOMUM AND *C. FLORIDA* (CORNACEAE)**

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Abstract.—Two *Rhagoletis* fruit parasites of dogwood, previously thought to be conspecific, are shown to be reproductively distinct. Laboratory studies show consistent differences in the flies' oviposition response to the respective host fruits and in production of viable F₁ hybrid first instar larvae. The specific status of the fly populations is discussed.

The four members of the *Rhagoletis pomonella* (Walsh) species group of true fruit flies (Diptera: Tephritidae) have been at the forefront of discussions concerning modes of host race formation and speciation. A sympatric model (Bush, 1969a) is based upon the assumption that mating and oviposition by each of these morphologically similar univoltine species of fruit parasites occur on plants in a different family. This model assumes that adult selection and larval use (larvae are confined to feeding on the fruit in which eggs were deposited) of a host plant are strongly affected by factors that are more similar within than among plant families and, therefore, that the recent colonization of a plant species in a novel family affords a considerable measure of reproductive isolation from the parental fly population. While the members of the *Rhagoletis suavis* (Loew) species group, for example, probably diverged in allopatry (now overlapping discontinuously in geographic range but entirely in host range [*Juglans* spp.: Juglandaceae]) and maintained reproductive integrity through variation in morphological characters involved with mate selection, divergence in host plant family association apparently provided the initial reproductive isolation that allowed for subsequent speciation of new host-associated populations from the ancestral form in the *R. pomonella* group (Bush, 1969b).

A reasonable prediction from the model above is that similar looking fruit flies attacking members of the same plant family are more likely to be conspecific than are those attacking heterofamilial plants. However, this appears to be contradicted by the existence of several populations of *Rhagoletis* flies on different dogwoods (*Cornus*: Cornaceae). *Rhagoletis electromorpha* Berlocher was recently described from flies that were reared from *C. drummondii* C. A. Meyer and *C. rasemosa* Lam. and were once considered conspecific with *R. tabellaria* (Fitch) flies collected from *C. stolonifera* Michx. (Berlocher, 1984) and *C. amomum* Mill. (Bush, 1966). A second example of host family overlap is provided by this study.

A population of *Rhagoletis* flies feeding on flowering dogwood (*Cornus florida* L.) was first noted by Benjamin (1934). The flowering dogwood fly is visually identical with *R. cornivora* Bush (the member of the *R. pomonella* sibling species group which infests *C. amomum*, *C. canadensis* L. [Bush, 1966] and *C. racemosa* [Smith, unpubl.]) and the two occur in partial sympatry in eastern North America (Bush, 1966). The logic of the sympatric speciation argument, as envisioned for this group of fruit flies,

dictates that these two *Cornus*-infesting flies are conspecific. However, electrophoretic enzyme analyses indicate that the flowering dogwood fly is much more closely related to *R. pomonella* than is *R. cornivora* (Berlocher, 1976; Smith, 1986). Although Berlocher (1976) suggested that these two dogwood flies were distinct, the data did not eliminate the possibility that differences in fruit chemistry may select for particular larval genotypes. This study demonstrates that *R. cornivora* and the flowering dogwood fly indeed are heterospecific, based on differences in reproductive biology related to host selection and to egg hatch success of test crosses.

MATERIALS AND METHODS

The *Rhagoletis* fruit parasites used in this study were collected from natural populations in eastern Illinois. *Rhagoletis cornivora* flies were from silky dogwood, *C. amomum*, in Champaign and flowering dogwood flies were from Fairfield. The *R. pomonella* flies used in the test of egg hatch success were collected in Urbana from downy hawthorn (*Crataegus mollis* [T. & G.] Scheele: Rosaceae). Infested fruits were placed in the laboratory on hardware cloth over moist vermiculite, in which exiting larvae pupated prior to spending diapause in cold storage at 5°C until needed. After removal from the cold, pupae were placed in an environmental chamber at 24°C, 50 ± 5% relative humidity and 20:4 (L:D) photoperiod. Flies were caged separately by population (and also by sex for those to be used in test crosses) and were provided with a constant source of water and food (enzymatic yeast hydrolysate and brown sugar). Flies were first tested when ca. 4 weeks old and presumably were sexually mature. Maturity was indicated by the continued presence of copulating pairs in mixed-sex cages, by male-male copulatory attempts in unisexual cages and by female (virgin and mated) oviposition into artificial fruit consisting of hollow black hemispheres of ceresin wax (Prokopy and Bush, 1973). Eggs were attached to the inner surface of the wax domes during oviposition, facilitating their detection and removal. Females from mixed-sex cages were assumed to be mated by the time that they were tested on fruit.

In the first procedure, 50 naive (no previous exposure to real fruit) mated females were selected randomly from each dogwood population and individually tested for oviposition response to single uninfested fruit clusters of freshly-picked *Cornum amomum* (5–10 fruit per cluster, each fruit ca. 5–10 mm diameter) and *C. florida* (3–6 fruit per cluster, each fruit ca. 8–12 mm diameter), whose stems were placed in a small vial of water to prevent dessication. Females were deprived of the wax dome oviposition substrates for 18 hours prior to fruit testing. Half of the test flies from each population were offered *C. amomum* first and *C. florida* second; the other half were offered fruits in the reverse order. Females were observed on the fruit until they oviposited into or left the fruit or until 15 minutes had elapsed without oviposition, in which case they were recorded as rejecting the fruit. Females were tested on the next fruit between 20–30 minutes after their previous exposure. All females were tested in only one trial sequence. The test result of each female was placed in one of the following categories of response to these two hosts: (a) accept only its own natural fruit; (b) accept only the other fly's natural fruit; (c) accept both fruits; (d) accept neither fruit. A female that oviposited into the first fruit but rejected the second was then offered the first fruit type again. If she rejected it this time, then she was recorded

as accepting neither fruit (response "d"). This was to ensure against a false negative response to the second fruit by a female that may have deposited her last mature egg into the first fruit and simply had no more available at that time for succeeding tests.

Rhagoletis eggs are deposited in punctures produced in the fruit by the extensible sclerotized ovipositor. Females sometimes either fail to deposit an egg in a puncture or fail to produce a puncture on a given attempt. Oviposition was suggested during observations when a female withdrew the ovipositor from the fruit puncture and dragged it over the fruit surface, depositing an oviposition-detering pheromone (Prokopy et al., 1976). However, oviposition was recorded only after fruit dissection revealed an egg. All punctures were dissected for eggs, even those made by non-dragging females.

In the second procedure, mated flies from each dogwood population were placed in clear plastic cylindrical fiberglass-screened cages (8 cm diameter, 10 cm tall) containing food, water and one mature freshly-picked Golden Delicious variety apple, *Malus pumila* Mill. (Rosaceae) (each fruit ca. 59–67 mm diameter). Each of the 20 cages per fly population contained one female and one male. At the end the first day, the apples were replaced and any ovipositor punctures were dissected for the presence of eggs. After two weeks of constant exposure to apples, another one-day count of eggs was obtained for each cage.

In the final procedure, 10 single-pair crosses of virgin flies from the two dogwood populations and from a hawthorn population of *R. pomonella* were performed for each of seven mating combinations (Table 1) in individual cages supplied with food, water and wax domes of 12 mm and 18 mm diameters. These domes were placed over moist cotton to reduce the chance of desiccation of eggs deposited into them. Only pairs observed in copulation for at least 15 minutes were used for egg hatch analysis. This copulation period is usually sufficient for successful sperm transfer in *R. pomonella* (Smith and McPheron, unpubl.). Males were left in the cages to permit additional mating during the period of egg collection, which lasted for 3 weeks for some cages. All pairs were casually observed in copulation at least twice more during this period. Eggs from each cage were removed daily from the inner surface of the domes with a fine brush and transferred to individual petri dishes containing moist filter paper. Hatching usually occurred within 2–6 days. If a larva did not develop within two weeks of egg collection, then the egg was recorded as unhatched. Data were subjected to analysis of variance and differences among means were determined by a Student-Newman-Keuls' test.

RESULTS AND DISCUSSION

In the measure of oviposition response to the two dogwood fruits, each fly population demonstrated fidelity to its natural host. Of the 50 females tested from each population, (a) 35 *R. cornivora* and 33 flowering dogwood flies oviposited only into their respective hosts, while (b) none accepted only the other fly's host. Only (c) two *R. cornivora* and six flowering dogwood flies accepted both fruits, while (d) 13 and 11, respectively, accepted neither fruit (see Methods for description of response category "d"). This host fruit fidelity generally agrees with similar tests (Smith, 1986) comparing flowering dogwood flies with *R. pomonella* flies from downy hawthorn.

Table 1. Mean proportions of hatched eggs from laboratory crosses of *R. cornivora* (cor), flowering dogwood flies (flo) and *R. pomonella* flies from hawthorn (haw). N = 10 for each mating combination. Means that are followed by the same letter are not significantly different by a Student-Newman-Keuls' test at P = 0.05.

Female × male	Eggs	Mean ± SEM
cor × cor	220	0.851 ± 0.043 a
cor × flo	213	0.023 ± 0.015 b
flo × cor	283	0.042 ± 0.014 b
flo × flo	318	0.940 ± 0.026 a
flo × haw	237	0.923 ± 0.029 a
haw × flo	287	0.919 ± 0.035 a
haw × haw	292	0.935 ± 0.031 a

However, a greater proportion of flowering dogwood fly eggs were deposited in the heterofamilial hawthorn fruit (ca. 43%) in that study than in the confamilial silky dogwood fruit (ca. 10%) here. A speculative explanation for this apparent anomaly follows from the observation that flowering dogwood flies oviposited into hawthorn fruit significantly more often than hawthorn flies did into flowering dogwood fruit (Smith, 1986). Perhaps the flowering dogwood fly descended from a hawthorn fly population and retained some degree of positive response to an ancestral host. Perhaps for the same reason, the apple-infesting population of *R. pomonella* responds even more strongly in the laboratory to its presumed ancestral hawthorn host (Walsh, 1867; Bush, 1966) than it does to apple itself (Prokopy et al., 1982; Smith, unpubl.).

In the oviposition assay with apples, none of the 20 cages of flies from either population had eggs after the first day. However, after two weeks of exposure to apple, 16 of 20 flowering dogwood fly cages yielded eggs (73 total) in the one day trial, but none of the *R. cornivora* cages did so. Thus, the flowering dogwood flies displayed a greater tendency to oviposit in apple than did *R. cornivora* flies (Mann-Whitney $U = 360$, two-tailed $P < .002$). Additional tests have shown that, even after long-term exposure to apples that have been artificially punctured with an insect pin, *R. cornivora* flies failed to oviposit in freshly-picked or in cold-stored apples and also spent little time on this relatively large fruit (Smith, 1986, and unpubl.). The results of fruit tests suggest that flowering dogwood flies may have (1) a lower threshold of induction for oviposition in novel fruit, in general, than do *R. cornivora* flies and/or (2) a lower threshold response to rosaceous fruit (e.g., apple and hawthorn), in particular.

The egg hatch results (Table 1) indicate that these two dogwood fly populations are not reproductively compatible. Interpopulational crosses produced far lower proportions of hatching eggs than did intrapopulational ones. The lower hatch success of *R. cornivora* eggs, which appear smaller than eggs of the other members of the species group, may result from a higher level of desiccation (or other damage) resulting from deposition into the wax domes. Thus, the overall proportion of *R. cornivora* × *R. cornivora* eggs that hatched may have been higher here (82% vs. 51%) than in Smith (1986) because of greater care here to reduce desiccation. As discussed in Smith

(1986), the specific mechanism(s) responsible for the reduction in heterospecific egg hatch is unknown.

The results of this study confirm the conclusion of Berlocher (1976) that the population of *Rhagoletis* flies on flowering dogwood is not conspecific with *R. cornivora*. Evidence from enzyme electrophoresis and from analyses of reproductive biology (mating propensity and egg hatch) suggest that this population is closely related to *R. pomonella* but still may be at least partly isolated from it by differences in host selection and seasonal availability (Smith, 1986). However, because the flowering dogwood flies tested were from an edge of the host's geographic range, these differences with *R. pomonella* from hawthorn may not be consistent with those from a more central (southern) part of the flowering dogwood range. Therefore, the flowering dogwood fly should be considered to be a host-associated population of *R. pomonella* at least until further population data become available.

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